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# Presenilin Affects Arm/β-Catenin Localization and Function in *Drosophila*

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***Presenilin* is an essential gene for development that when disrupted leads to a neurogenic phenotype that closely resembles Notch loss of function in *Drosophila.* In humans, many naturally occurring mutations in *Presenilin 1* or *2* cause early onset Alzheimer's disease. Both loss of expression and overexpression of *Presenilin* suggested a role for this protein in the localization of Armadillo/β-catenin. In blastoderm stage *Presenilin* mutants, Arm is aberrantly distributed, often in Ubiquitin-immunoreactive cytoplasmic inclusions predominantly located basally in the cell. These inclusions were not observed in loss of function *Notch* mutants, suggesting that failure to process Notch is not the only consequence of the loss of *Presenilin* function. Human *presenilin 1* expressed in *Drosophila* produces embryonic phenotypes resembling those associated with mutations in *Armadillo* and exhibited reduced Armadillo at the plasma membrane that is likely due to retention of Armadillo in a complex with Presenilin. The interaction between Armadillo/β-catenin and Presenilin 1 requires a third protein which may be δ-catenin. Our results suggest that Presenilin may regulate the delivery of a multiprotein complex that regulates Armadillo trafficking between the adherens junction and the proteasome.** 

***Key Words:*** **presenilin; PS1; β-catenin; Armadillo; δ-catenin; cell adhesion; Alzheimer's disease; Notch; Wingless; *Drosophila.***

## INTRODUCTION

*Presenilin 1 (PS1)* is the gene most commonly mutated in familial Alzheimer's disease with over 50 different mutations described. All reported mutations lead to an Alzheimer phenotype that is clinically and pathologically indistinguishable from sporadic disease except for the early age of onset (reviewed in Ray *et al.*, 1998). Mutations in the *PS1* paralog *PS2* also cause Alzheimer's disease (AD), although these are much less frequent. The Presenilins (PS) are polytopic membrane proteins that undergo endoproteolytic cleavage into two stable fragments, which remain associated with each other (Thinakaran *et al.*, 1996). These proteins are closely linked to an activity called γ-secretase which is responsible for the intramembranous cleavage and subsequent release of the Aβ peptide from the amyloid precursor protein (APP). Support for linking PS to γ-secretase activity comes from the observed reduction in secreted Aβ in the absence of PS1 (De Strooper *et al.*, 1998), the total inactivation of the γ-secretase activity in cells which lack both PS1 and PS2 (Herreman *et al.*, 2000; Zhang *et al.*, 2000), the universal increase in Aβ levels in the presence of presenilin missense mutations (Borchelt *et al.*, 1996; Citron *et al.*, 1997; Duff *et al.*, 1996; Scheuner *et al.*, 1996; Tomita *et al.*, 1997; Xia *et al.*, 1997), the requirement of two PS transmembrane aspartate residues for γ-secretase activity (Wolfe *et al.*, 1998), and transition-state analogue inhibitors which bind directly and specifically to PS1 heterodimers (Esler *et al.*, 2000; Li *et al.*, 2000). The increased secretion of Aβ associated with PS mutations leads to increased deposition of amyloid found in AD senile plaques (Lemere *et al.*, 1996; Mann *et al.*, 1996).

PS1 serves a crucial function in vertebrate development as demonstrated by targeted disruption of the *PS* genes in the mouse, which results in an embryonic lethal phenotype with defects in somite segmentation and differentiation (Donoviel *et al.*, 1999; Shen *et al.*, 1997; Wong *et al.*, 1997). This phenotype resembles that observed with loss of the *Notch-1* gene (Conlon *et al.*, 1995; Swiatek *et al.*, 1994). Likewise, genetic null mutations of the *Drosophila PS*

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(*DPS*) gene results in a neurogenic defect (Struhl and Greenwald, 1999; Ye *et al.*, 1999) and *in vitro* assays (De Strooper *et al.*, 1999) suggest that an activity associated with Presenilin cleaves Notch to release the intracellular domain which then translocates to the nucleus. These findings support earlier suggestions of an interaction between Notch and PS1 (Levitan and Greenwald, 1995; Ray *et al.*, 1999).

A second family of key developmental proteins, members of the Arm-repeat family, are also associated with Presenilin (Murayama *et al.*, 1998; Tesco *et al.*, 1998; Yu *et al.*, 1998; Zhang *et al.*, 1998; Zhou *et al.*, 1997). Armadillo (Arm)/β-catenin, the prototypical member of this family, binds to classical cadherins and to the actin cytoskeleton via α-catenin to form an adherens junction complex (Aberle *et al.*, 1994; Oyama *et al.*, 1994). Arm/β-catenin is also involved in the Wnt/Wg cell determination pathway. Signaling via the Wnt/Wg ligand stabilizes β-catenin, allowing for its translocation to the nucleus bound to T cell factor–lymphoid enhancer factor (TCF/Lef1) (Brunner *et al.*, 1997; Riese *et al.*, 1997; van de Wetering *et al.*, 1997). Other members of the Arm-repeat family also interact with PS1 (Levesque *et al.*, 1999; Stahl *et al.*, 1999; Zhou *et al.*, 1997). These family members are more distantly related to β-catenin (Peifer *et al.*, 1994) with only 10 Arm repeats (instead of 13 in the case of β-catenin) and with highly divergent amino and carboxy terminal extensions flanking the Arm-repeat domain. One of these interactors, δ-catenin, is expressed specifically in the nervous system (Ho *et al.*, 2000; Zhou *et al.*, 1997) and may contribute to the severe mental retardation of Cri-du-Chat syndrome when haploinsufficient (Medina *et al.*, 2000). Other members of this family include $p120^{ctn}$ (Peifer *et al.*, 1994) and p0071 (Hatzfeld and Nachtsheim, 1996), which has also been shown to interact with PS1 (Stahl *et al.*, 1999). In contrast to β-catenin which binds to the extreme carboxy terminus of several cadherins, members of the $p120^{ctn}$ subfamily including δ-catenin bind to the juxtamembrane region of the cadherins (Lu *et al.*, 1999; Ozawa and Kemler, 1998; Yap *et al.*, 1998). It is notable that not all Arm-repeat family members interact with PS1, as plakoglobin does not interact with PS1 (Levesque *et al.*, 1999). In this study we show that β-catenin and δ-catenin have distinct PS1 binding characteristics.

The study of how *PS1* mutations may affect the binding and function of Arm-repeat family members within the complex has generated experimental support for the idea that *PS* mutations may retard β-catenin translocation to the nucleus (Nishimura *et al.*, 1999) and decrease β-catenin stability (Zhang *et al.*, 1998). Curiously, *PS1* mutations have also been reported to increase β-catenin stability by failing to recruit GSK-3β into the PS1–β-catenin complex (Kang *et al.*, 1999). These conflicting data prompted a search for an experimental approach in which the functional consequences of the interactions with Arm family members might be demonstrated. Expression of human PS1 in *Drosophila* embryos using the GAL-4/UAS expression system revealed specific phenotypes with a common feature: all failed to localize Armadillo to the cell membrane and consequently manifested severe disorganization of cytoskeletal components and cell adhesion defects. When embryos derived from females lacking *Drosophila PS1* were examined, Armadillo was aberrantly localized in Ubiquitin-positive cytoplasmic inclusions. These observations suggest that regulation of PS1 activity is important for the proper intracellular localization of Arm.

## RESULTS

### *Loss of Presenilin Affects Localization of Armadillo/β-Catenin*

To investigate the functional significance of the previously reported association between PS and β-catenin, localization of Arm in embryos derived from females with *DPS* homozygous mutant germlines were examined. These embryos, referred to as "*DPS* mutant embryos" develop in the complete absence of maternally contributed *DPS* mRNA and therefore lack DPS protein during early developmental stages. At the cellular blastoderm stage in wild-type embryos, Arm is apically localized at the membrane outlining the hexagonal array of cells (Fig. 1A). At a similar stage in *DPS* mutant embryos, Arm is observed at the plasma membrane, but not in the sharply defined manner observed in wild-type embryos (compare Fig. 1A to 1B). Further, there are large cytoplasmic inclusions of Arm in the *DPS* mutant embryos (Fig. 1D) which are predominantly localized to the basal-most region of the cells at the blastoderm stage and are not observed in wild-type embryos (Fig. 1C). These inclusions can also be observed at slightly later stages, at which time some cytoplasmic Arm is present in basal inclusions (yellow arrows; Fig. 1E) as well as apically and at the adherens junctions (white arrowheads, Fig. 1E). This observation suggests that DPS is required for proper or efficient localization of Arm to the apical membrane in the cellular blastoderm. It should be noted, however, that enough of the very abundant Arm protein reaches the adherens junctions to support cell adhesion, as demonstrated by the fact that embryos from these *DPS* germline clone mothers do not exhibit the loss of cell adhesion phenotype, but rather ultimately display a neurogenic phenotype (Struhl and Greenwald, 1999; Ye *et al.*, 1999).

We next sought to investigate the composition of the cytoplasmic Arm inclusions. One possibility was that these inclusions were ubiquitinated Arm protein sent to the proteosomes for degradation. Antibodies directed against Ubiquitin colocalized with some of the Arm inclusions observed in *DPS* mutant embryos (Figs. 1F–1H). Double labeling revealed that much of the Arm found in cytoplasmic inclusions contained Ubiquitin (Fig. 1H). In slightly older blastoderm embryos (Figs. 1I–1K), the amount of Ubiquitin and Arm colocalization in cytoplasmic inclusions appears to increase (compare Fig. 1G with 1J). However, some Ubiquitin-positive inclusions did not contain Arm, which suggests that other proteins may also fail to

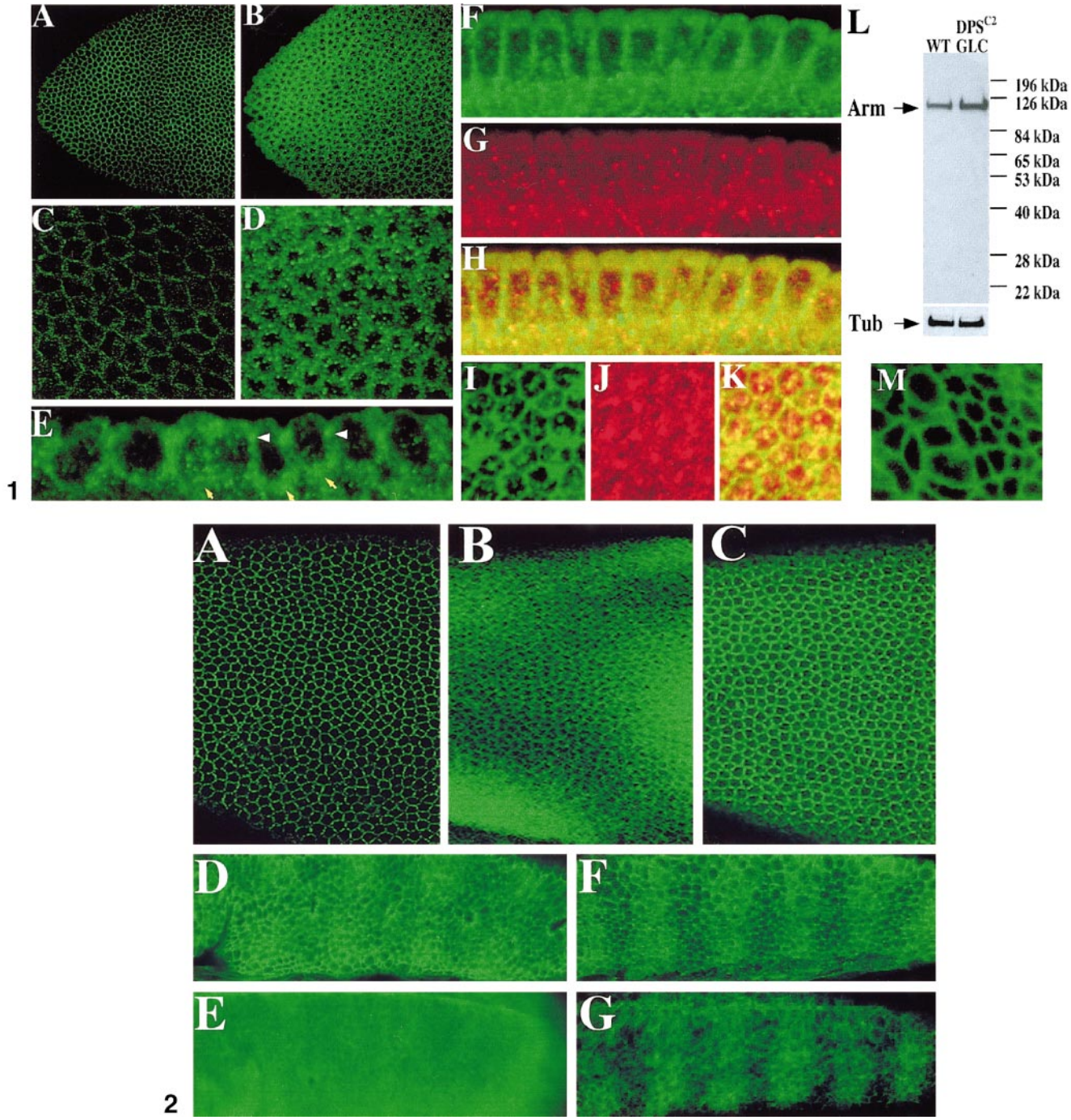


**FIG. 1.** Aberrant localization of Armadillo in null mutation DPS embryos. In blastoderm stage wild-type embryos stained with antibodies directed against Arm (A, 20×), a hexagonal array of cells can be seen. Arm is tightly associated with cell membranes and little appears in the cytoplasm. In embryos derived from $DPS^{C2}$ GLCs, Arm expression is not as tightly restricted to the cell membrane, and there is punctuate staining in the cytoplasm (B, 20×). A basal focal plane of a wild-type cellular blastoderm (C, 40×) illustrates the limited amount of Arm normally found basally in the cell, whereas in $DPS^{C2}$ embryos large cytoplasmic inclusions are observed basally. (D, 40×) In a germband extended stage $DPS^{C2}$ embryo (E, 100×), some Arm is present apically at the adherens junctions (white arrowheads), but most is found basally and is not tightly associated with the cell membrane (yellow arrows). In early blastoderm stage $DPS^{C2}$ embryos double labeled with anti-Arm (FITC, F) and anti-Ubiquitin (Texas red, G; merged image, H) some of the cytoplasmic Arm colocalized with Ubiquitin (yellow and orange in H). Toward the end of blastoderm stages, increasing amounts of basally located, cytoplasmic Arm colocalized with Ubiquitin [yellow and orange in K (Arm FITC, I; Ubiquitin TR, J; merged image, K)]. However, most of the Arm tightly associated with the

reach the proteosome in DPS mutant embryos. If the cytoplasmic inclusions of Arm are due to a failure to undergo appropriate degradation, then the amount of Arm found in the DPS embryos would be expected to be increased. Immunoblots of equal number of embryos revealed a 2.5-fold increase in the total amount of Armadillo in germline clones (Fig. 1L), while a loading control (Tub) demonstrated that both lanes were equally loaded. These findings suggest that DPS may function in proper proteasomal degradation of cytoplasmic Arm during blastoderm stages. It should be noted that when embryos carrying a deficiency for the *Notch* locus were stained with Arm, no cytoplasmic inclusions were observed at any stage (Fig. 1M). Although we did not observe any Arm inclusions in *Notch*-deficient embryos, even at late embryonic stages, it is formally possible that residual Notch from maternally loaded mRNA is sufficient to prevent the inclusion formation observed in *DPS* mutant embryos.

### *Expression of PS1 Affects Arm Membrane Localization and Accumulation*

To investigate further the interaction between Arm and DPS *in vivo*, we expressed human PS1 full-length protein (hPS1) or the loop region alone (hPS1loop), during embryogensis using the Gal4/UAS system (Brand and Perrimon, 1993). When expressed using a strong maternal Gal4 driver (*nanos-Gal4*) the vast majority of embryos were unable to maintain cellular integrity and disintegrated. Interestingly, in the embryos that survived, Arm localization was abnormal. However, the nature of the mislocalization was quite different from that observed in the *DPS* mutant embryos. In control embryos (*nanos-Gal4/+*) almost all Arm was associated with the cell membrane at blastoderm stages (Fig. 2A). But in *UAS-hPS1/+; nanos-Gal4/+* and *UAS-hPS1loop/+; nanos-Gal4/+* embryos, the appearance of the hexagonal array was "fuzzy" (making the images appear out of focus), due to greatly increased levels of cytoplasmic Arm (Figs. 2B and 2C). Although, qualitatively the mislocalization of Arm in the cytoplasm was the same with expression of either hPS1 or hPS1loop (compare Fig. 2B with 2C), both insertions of *UAS-hPS1* gave a much stronger phenotype than those associated with the *UAS-hPS1loop* insertions. This difference suggested that the physical interaction between hPS1loop and Arm is less robust than between the full-length hPS1 and Arm.

To confirm the observation that Arm is mislocalized to the cytoplasm in the presence of high levels of hPS1 or hPS1loop, we used another maternally expressed Gal4 driver (*α-tubulin-Gal4*). The expression of this Gal4 driver is somewhat weaker than the *nanos-Gal4*, thus allowing us to examine the effects of hPS1 or hPS1loop expression at later embryonic stages. Early in germband extension, *UAS-hPS1/+; α-tubulin-Gal4/+* (Fig. 2E) or *UAS-hPS1loop/+; α-tubulin-Gal4/+* (data not shown) embryos exhibited very high levels of cytoplasmic Arm across the entire segment, obscuring the visualization of the cell membranes. At this stage, the evolving parasegmental boundaries which are beginning to be evident in WT embryos (Fig. 2D) can hardly be seen at all in *UAS-hPS1/+; α-tubulin-Gal4/+* (Fig. 2E). As germband retraction proceeds, the normal stabilization of Arm in cells receiving Wingless signal occurred in *UAS-hPS1/+; α-tubulin-Gal4/+* but Arm associated with the membrane remained depleted across the parasegment, making the cell membranes appear indistinct (compare Fig. 2F to Fig. 2G).

### *Expression of hPS1 Generates Cadherin-like Mutant Phenotypes*

Arm at the cell membrane is associated with E-cadherin, and the continued expression and function of E-cadherin are dependent on the presence of functional Arm. E-cadherin is encoded by the *shotgun* gene, and *shotgun* mutant embryos develop poorly formed cuticles due to a loss of cell adhesion (Figs. 3B, 3C, and 3F). We reasoned that, if the increased level of cytoplasmic Arm observed in the presence of overexpression of hPS1 correlated with a depletion of the

membrane does not appear to be ubiquitinated (green in H and K), and not all the Ubiquitin-positive inclusions in the cytoplasm contain Arm (red in H and K). (L) Immunoblot analysis of lysates from an equal number of embryos from wild-type and $DPS^{C2}$ germline clones with an anti-Armadillo antibody. (Bottom panel) The same samples blotted with an anti-tubulin antibody as a loading control, which demonstrates that the lanes are equally loaded and the increased amount of Arm found in the $DPS^{C2}$ embryos is not due to a loading artifact. Notch mutant embryos do not exhibit the cytoplasmic inclusions of Arm (M).

**FIG. 2.** Overexpression of Presenilin affects the intracellular localization of Arm. Cellular blastoderm stage embryos (20×) stained with Arm antibody in wild-type [*nanos-Gal4/+*] (A), *nanos-Gal4/+; UAS-PS1*$^{D2}$*/+* (B), and *nanos-Gal4/+; UAS-PS1-loopA/+* (C). Note that most Arm is localized to the membrane and there is very little in the cytoplasm in wild-type cellular blastoderm stage embryos (A), giving a very sharply defined, hexagonal pattern of cells. When full-length hPS1 (B) or the loop region of hPS1 (C) is expressed using the same Gal4 driver, the cytoplasmic pools of Arm are increased and the hexagonal array of cells normally outlined by Arm associated with the membrane are less apparent. Armadillo expression in *α-tubulin-Gal4* alone (D, F; 20×) or in combination with *UAS-PS1*$^{D2}$ (E, G; 20×). Early in germband extension cytoplasmic clearing of Arm from the anterior portion of each segment occurs in cells that do not respond to Wingless (D). In embryos where hPS1 (E) or hPS1loop (not shown) is overexpressed, this anterior cytoplasmic clearing of Arm is not evident, and the cell membranes are difficult to identify. At later stages membrane-associated Arm outlines the cells in the anterior segment in wild type (F), but is less distinct in animals expressing hPS1(G) or the loop region (not shown).

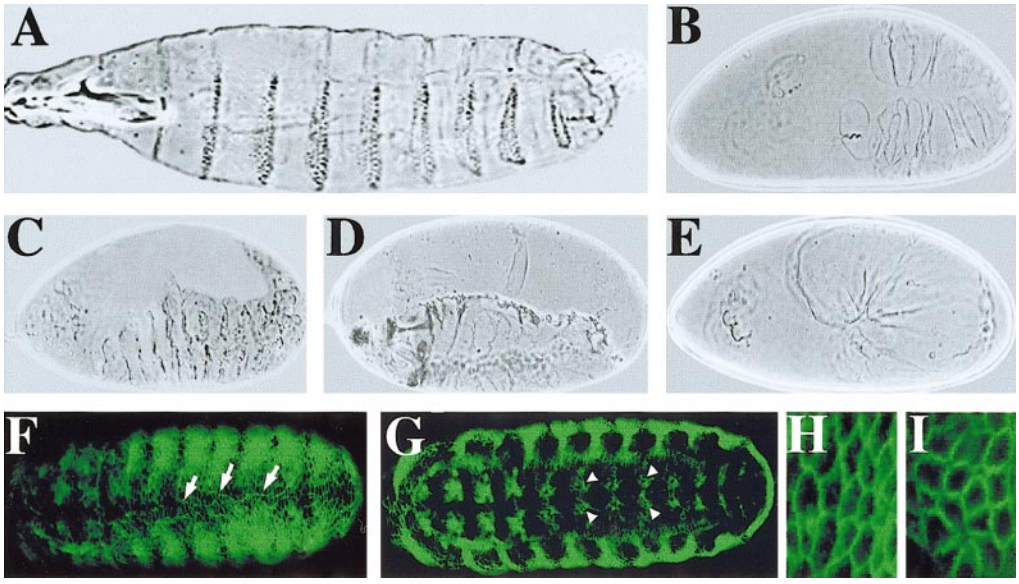


**FIG. 3.** Effects of overexpression of hPS1 or the loop region during early gastrulation. The effect of loss of Arm at the membrane due to overexpression of hPS1 or the loop region alone is clearly apparent in the larval cuticle. (A) A wild-type first-instar larva with the segmentally reiterated pattern of denticle bands and normal head and tail structures. (D and E) The ventral cuticles of *α-tubulin-Gal4/+; UAS-PS1-loopA/+* and *α-tubulin-Gal4/+; UAS-PS1D4/+*, respectively. These phenotypes resemble the cuticle phenotypes observed in $shg^{G317}$ (C) and $shg^{G3085}$ (B) mutant embryos. *shg* alleles are mutations in *Drosophila E-cadherin,* which result in a loss of cell adhesion. (F, G, and I) *α-tubulin-Gal4/+; UAS-PS1*$^{D2}$*/+* embryos nearing completion of gastrulation stained with an antibody to Fas III. (F) A ventral view of the ectoderm demonstrating that the ventral ectoderm is both present and intact at the midline (arrows), although the midline wanders slightly due to the mild perturbations of segmentation that are seen in animals that live to this stage. When the nervous system is visualized in the same embryo (G), the appropriate number of cells in the nervous system are labeled (arrows) and the general organization of the nervous system appears normal, indicating that these embryos are not neurogenic. When the cellular localization of Fas III in the ectoderm in *α-tubulin-Gal4/+; UAS-PS1*$^{D2}$*/+* embryos (I) is compared to wild type (H; *α-tubulin-Gal4/+*) no mislocalization is apparent as is the case for Arm.

membrane-associated pool of Arm, then *UAS-hPS1loop/+; α-tubulin-Gal4/+* should exhibit a *shotgun*-like cuticle phenotype. Indeed most of the *α-tubulin-Gal4/+; UAS-hPS1/+* (Fig. 3E) or the *α-tubulin-Gal4/+; UAS-hPS1loop/+* embryos (Fig. 3D) that survive long enough to secrete cuticle exhibit phenotypes that resemble the loss of E-cadherin function. In embryos that develop more cuticular elements, additional phenotypes include loss of head structures, variable degrees of segmental fusion, and a "dorsal open" phenotype. In a small subset of animals (<10%) that survive to secrete cuticle, a weak/moderate *wingless*-like cuticle phenotype is observed, and this phenotype can be correlated with a failure to maintain Engrailed expression (data not shown).

Because DPS appears to have a role in the Notch pathway, α-tubulin-Gal4/+; UAS-hPS1/+ embryos were labeled with Fas III to assess the integrity of the ventral ectoderm and to look for any indication of hypertrophy of the nervous system. When the ectodermal cells associated with the ventral midline were examined there was evidence of some disruption in patterning as indicated by the meandering of the midline (arrows, Fig. 3F), but the epidermis was found to be intact. Further, when the Fas III-positive cells in the nervous system of these same embryos were visualized we found that the number and location of Fas III-positive cells were normal (Fig. 3G). This indicates that the nervous system hypertrophy at the expense of ventral ectodermal cell fates that is found as a result of disruption in the Notch signaling pathway does not occur when UAS-hPS1 is expressed.

Since Fas III can be found at the cell membrane we examined the localization of Fas III in α-tubulin-Gal4/+; UAS-hPS1/+ (Fig. 3I) and in α-tubulin-Gal4/+; UAS-hPS1loop/+ embryos (data not shown). Compared to wild-type (Fig. 3H), there was no obvious mislocalization of Fas III as a result of UAS-hPS1 expression (Fig. 3I). This suggests that the mislocalization of Arm as a result of UAS-hPS1 expression is not due to some nonspecific effect, but rather reflects a specific interaction been Arm and hPS1.

### *PS1 Overexpression Results in a "Dorsal Open" Phenotype*

The resemblance of the cuticle phenotypes associated with hPS1 and hPS1loop expression to shotgun mutations strongly implicates dysfunction in adhesion and cytoskel-

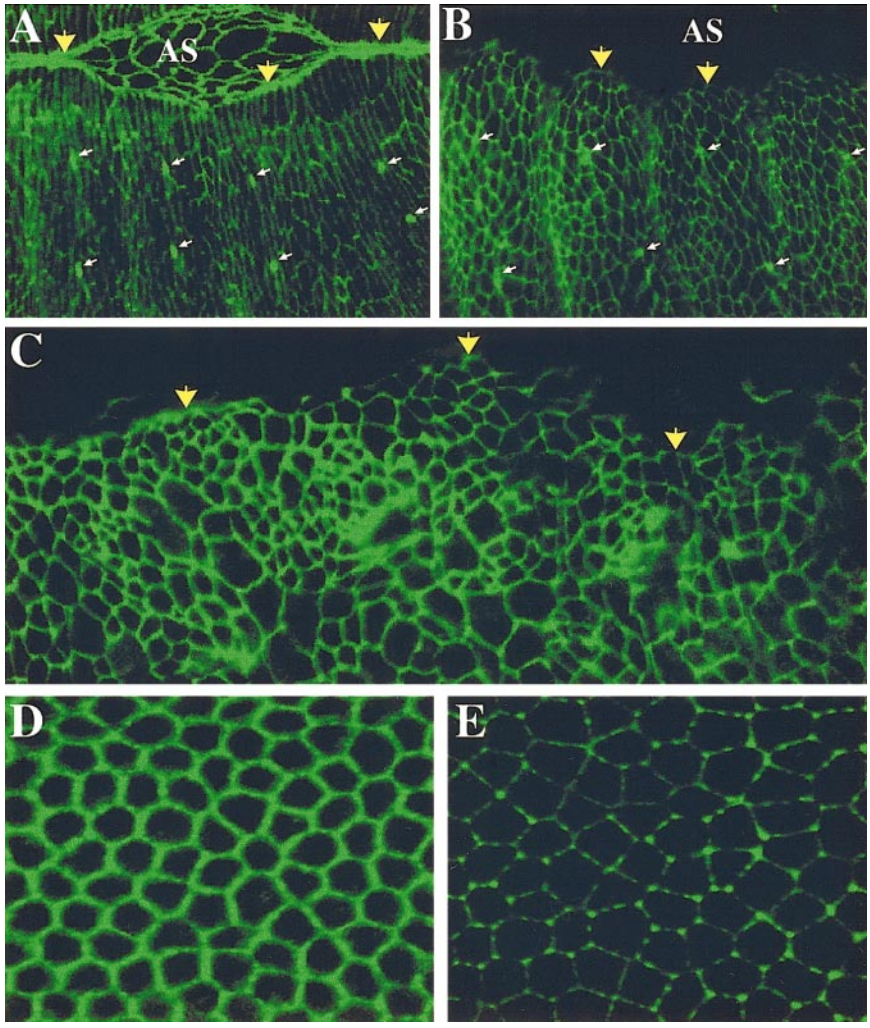


**FIG. 4.** Dorsal closure defects and effects on actin. Phalloidin staining in *α-tubulin-Gal4* alone (A, D) or in combination with *UAS–PS1D2* (B, C, E). There is an accumulation of actin at the leading edge of the dorsal ectoderm as it elongates (yellow arrows in A), internalizing the cells of the amnioserosa (AS). As dorsal closure nears completion, there is also an accumulation of actin associated with differentiating structures in the peripheral nervous system (white arrows in A, B). In *α-tubulin-Gal4/+; UAS-PS1D2/+* dorsal closure does not occur, the ectodermal cells do not elongate, and there is no accumulation of actin at the leading edge (yellow arrows in B, C). Note that the accumulation of actin associated with differentiating structures in the PNS is apparent (white arrows in B), which indicates that this embryo should be approaching completion of dorsal closure. At the blastoderm stage, phalloidin staining allows visualization of the hexagonal array of cells by labeling actin associated with the cell membrane (D). However, in *α-tubulin-Gal4/+; UAS-PS1D2/+* animals the pattern of phalloidin staining becomes very punctuate, and there is a dramatic reduction in the amount of actin associated with the membrane (E).

etal organization. To investigate the basis of this phenotype further we examined phalloidin staining in these embryos at stages before cuticle deposition. Actin associated with the cytoskeleton is important both for cellularization and for the changes in cell shape during dorsal closure at midembryonic stages. During normal development the dorsal-most cells of the epidermis, which are initially located on the lateral side of the embryo, change from a generally rounded shape to a more cuboidal shape (Knust, 1996; Young *et al.*, 1991, 1993) and are known as the "leading edge." After altering their shape, the leading edge cells serve an instructive role in directing the more lateral cells of the epidermis to undergo a similar change in shape and a stretching that facilitates the advancement of the epidermis over the cells of the amnioserosa, which becomes internalized. The leading edge cells have an enrichment of actin along their dorsal-most edge that is maintained as they contact their counterparts that have advanced from the opposite side of the embryo (yellow arrows in Fig. 4A). Leading edge cells contact each other initially anteriorly

and posteriorly, and dorsal closure then proceeds from both ends toward the middle of the embryo.

In UAS-hPS1/+; α-tubulin-Gal4/+ (Figs. 4B and 4C) and UAS-hPS1loop/+; α-tubulin-Gal4/+ (data not shown) animals that make it through cellularization usually fail to initiate and/or complete dorsal closure, a phenotype resembling that observed in some Arm mutations (Peifer *et al.*, 1991). At the stage when an accumulation of actin in the peripheral nervous system is clearly apparent (white arrows in Figs. 4A and B), dorsal closure should be approaching completion. However, in the UAS-hPS1/+; α-tubulin-Gal4/+ animals, the leading edge cells do not undergo the proper change in shape (yellow arrows in Fig. 4B), and there is no accumulation of actin along the dorsal most edge of the cells (yellow arrows in Fig. 4C). Note that cells all along the dorsoventral axis of the epidermis fail to stretch and take on the proper thin, cuboidal shape (Fig. 4B), as occurs in the wild type (Fig. 4A). In the more extreme cases the epidermal cells are often very irregularly shaped with little apparent organization along the anterior–posterior axis (Fig. 4C).

Alterations in the normal distribution of actin due to expression of hPS1 or the loop region can also be observed in blastoderm stage embryos, concurrent with the mislocalization of Arm to the cytoplasm. Wild-type phalloidin staining resembles the pattern of Arm staining at blastoderm stages, revealing the hexagonal array of cells (Fig. 4D). However, in UAS-hPS1/+; α-tubulin-Gal4/+ animals, the overall amount of actin present at the membrane is greatly reduced, resulting in a thin, spotty phalloidin staining pattern in some regions of the embryo (Fig. 4E) and often in a complete degeneration of the membrane structure in other regions (data not shown). In areas where hexagonal arrays are not present, large patches of intense phalloidin staining are evident.

### β-Catenin Does Not Interact Directly with PS1 in Vitro

Our *in vivo* data support the model that PS1 and Arm/β-catenin interact and are consistent with previous observations that PS1 and β-catenin associate in various cell lines and brain tissues (Murayama *et al.*, 1998; Nishimura *et al.*, 1999; Tesco *et al.*, 1998; Yu *et al.*, 1998; Zhang *et al.*, 1998; Zhou *et al.*, 1997) and that the interaction requires the PS1 CTF (Murayama *et al.*, 1998; Tesco *et al.*, 1998). However, because all of the studies describing this interaction rely on either coimmunoprecipitation or cofractionation assays, it is unknown whether the interaction between PS1 and β-catenin is direct or requires additional factor(s). To address this issue, different mouse β-catenin deletion constructs were fused to the GAL4 AD in the yeast vector pACT2 and tested against the human PS1 loop fused to the GAL4 BD (pAS2loop; amino acids 263–408) (Zhou *et al.*, 1997). Surprisingly, none of those constructs specifically interacted with the hydrophilic loop of human PS1 (Figs. 5A and 5B).

One explanation for this observation would be that none of our constructs contained the intact complete binding domain. However, additional *in vitro* experiments made this explanation unlikely. Using [$^{35}$S]Met-labeled, *in vitro* translated full-length mouse β-catenin and either full-length PS1 or PS1 loop proteins, we failed to detect complex formation in coimmunoprecipitation assays with specific antibodies. PS1 failed to coimmunoprecipitate with β-catenin antibodies and β-catenin did not coimmunoprecipitate with PS1 antibodies (data not shown). Taken together, these observations suggest that some cofactor or posttranslational modification is required for β-catenin to bind to PS1, and this cofactor is not present in the yeast two-hybrid or coimmunoprecipitation assays. Notable is a recent report mapping the binding site to residues 445–676 of β-catenin by coimmunoprecipitation analysis of deletion constructs (Murayama *et al.*, 1998). However, our pACT–BC6 construct (residues 423–709) includes that region and yet still failed to bind in the two hybrid assay. Consistent with these findings R. Cavallo and M. Peifer (personal communication) also failed to observe a direct interaction between the *Drosophila* orthologues of β-catenin and PS1 using a LexA-based yeast two-hybrid system.

### β- and δ-Catenin Can Compete for Binding to PS1

Because δ-catenin binds directly to PS1, we asked whether δ-catenin could outcompete β-catenin from an *in vivo* complex with PS1 in CHO cells. For these experiments we used a small fragment of δ-catenin (ΔEco) identified by a two-hybrid analysis in which a series of δ-catenin truncations were tested for their ability to interact with PS1 (M. Medina and K. S. Kosik, unpublished data). The minimal interactive fragment requires both the last four Arm repeats and a portion of the carboxy terminal sequence just beyond the Arm repeats. The ΔEco fragment was cloned under the control of the CMV promoter and transfected into CHO–PS70 cells. Lysates from transfected cells were immunoprecipitated with antibodies against PS1 or β-catenin and the immunoprecipitates analyzed by immunoblot using an anti-β-catenin antibody. As shown in Fig. 5C, expression of the δ-catenin ΔEco fragment (residues 828–1127) almost completely displaced β-catenin from the PS1 complex.

These experiments support the hypothesis that β-catenin interacts with the hydrophilic loop of PS1 via a third protein, which competes with the ΔEco fragment of δ-catenin for binding to PS1. The most parsimonious explanation for these observations is that β-catenin associates with the complex via full-length δ-catenin itself, but was unable to do so in the presence of the ΔEco fragment alone. If this were the case, then full-length δ-catenin would not be expected to compete β-catenin from the PS1 complex, as the ΔEco fragment of δ-catenin did. Furthermore, it should be possible to demonstrate a direct interaction between δ-catenin and β-catenin. Indeed, in the presence of full-length δ-catenin, β-catenin is retained in the PS1 complex, suggesting that δ-catenin is capable of mediating β-catenin association with PS1 (Fig. 5C).

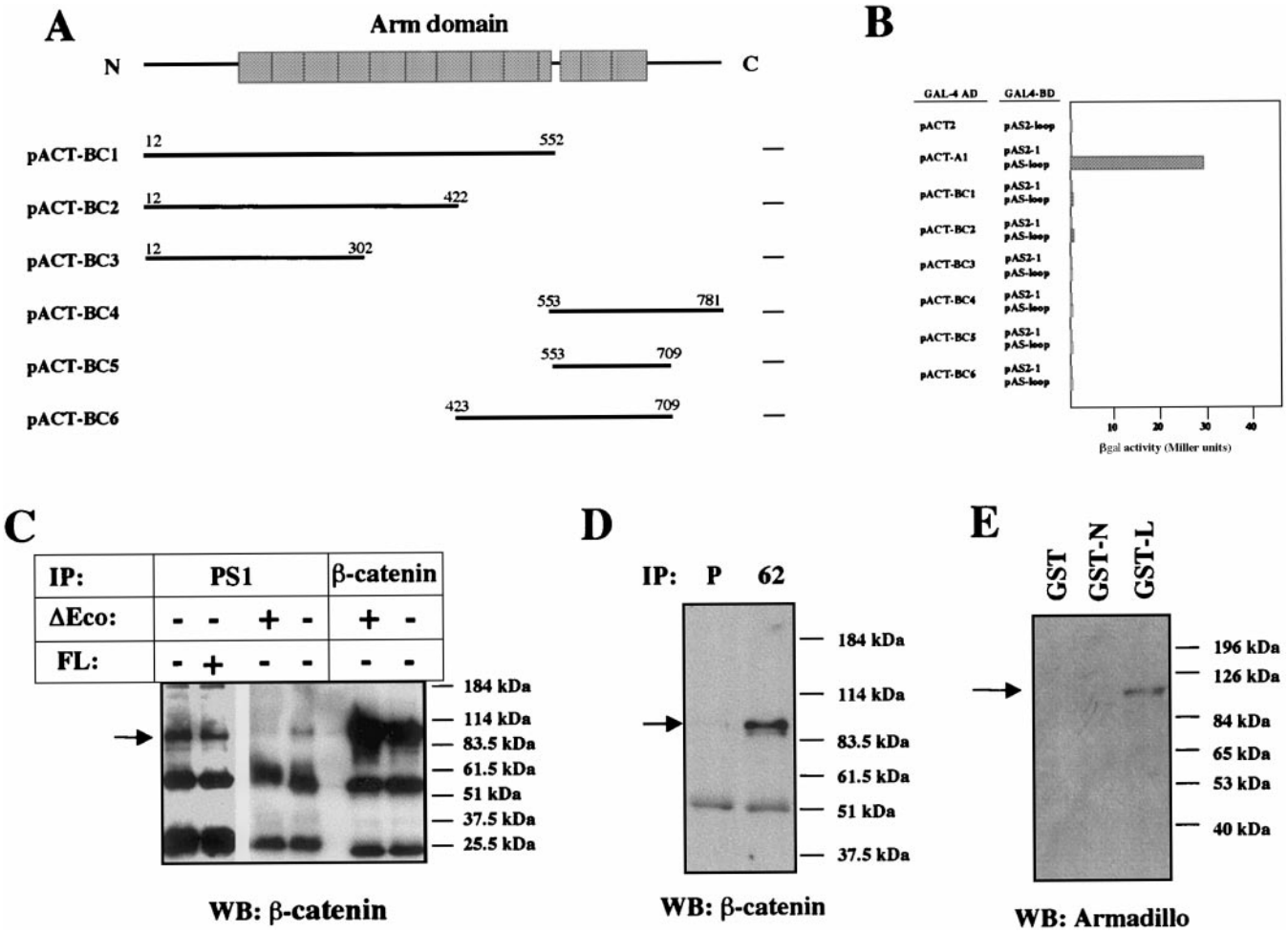


**FIG. 5.** β-Catenin does not bind directly to PS1. (A) Schematic summary of the mouse β-catenin fragments and their interaction with PS1. All β-catenin fragments were cloned into pACT2 and cotransformed with the pAS2-loop bait vector. The thick black line on the top represents the full-length β-catenin molecule. The 13 Arm repeats are shown as dark boxes. Amino acid residue numbers are shown on top of the fragments. (B) β-Galactosidase activity was determined in liquid assays to quantify each interaction. Plasmid pACT2 containing the GAL4–BD alone was used as a control of specificity. A δ-catenin fragment (pACT-A1) was used as a positive control. (C) Detection of β-catenin by immunoblot analysis of PS1 (antibody 4873) and β-catenin immunoprecipitates from CHO–PS70 cells transfected with the full length δ-catenin (FL) or the ΔEco fragment. (D) Detection of β-catenin in δ-catenin (antibody 62) and preimmune serum (P) immunoprecipitates from an adult rat brain lysate. Arrows in C and D point to β-catenin. (E) Wild-type *Drosophila* embryo lysates were affinity precipitated with GST alone, fused to the human PS1 N-terminus (N) or the loop region (L), and then analyzed by immunoblot with an anti-Armadillo antibody. Arrow indicates the Arm-immunoreactive band.

## DISCUSSION

### *The Localization of Armadillo Depends on Presenilin*

Embryos derived from *presenilin* germline clone females exhibited mislocalization of Armadillo. These embryos contained cytoplasmic inclusions that were both Arm and Ubiquitin immunoreactive, suggestive of a failure to target Arm to a degradative pathway. A role for PS in regulating the degradation of proteins is suggested by other PS interactions. The *C. elegans* orthologue of PS, sel-12 interacts with sel-10, a member of the Cdc4p family which targets proteins for Ubiquitin-mediated turnover (Wu *et al.*, 1998). Furthermore, the fly orthologue of Cdc41p, Slimb, may target β-catenin for Ubiquitin/proteasome degradation (Jiang and Struhl, 1998). Also, the LEF/β-catenin complex is thought to be affected in its translocation to the nucleus by mutations in PS (Nishimura *et al.*, 1999). A genetic rela-

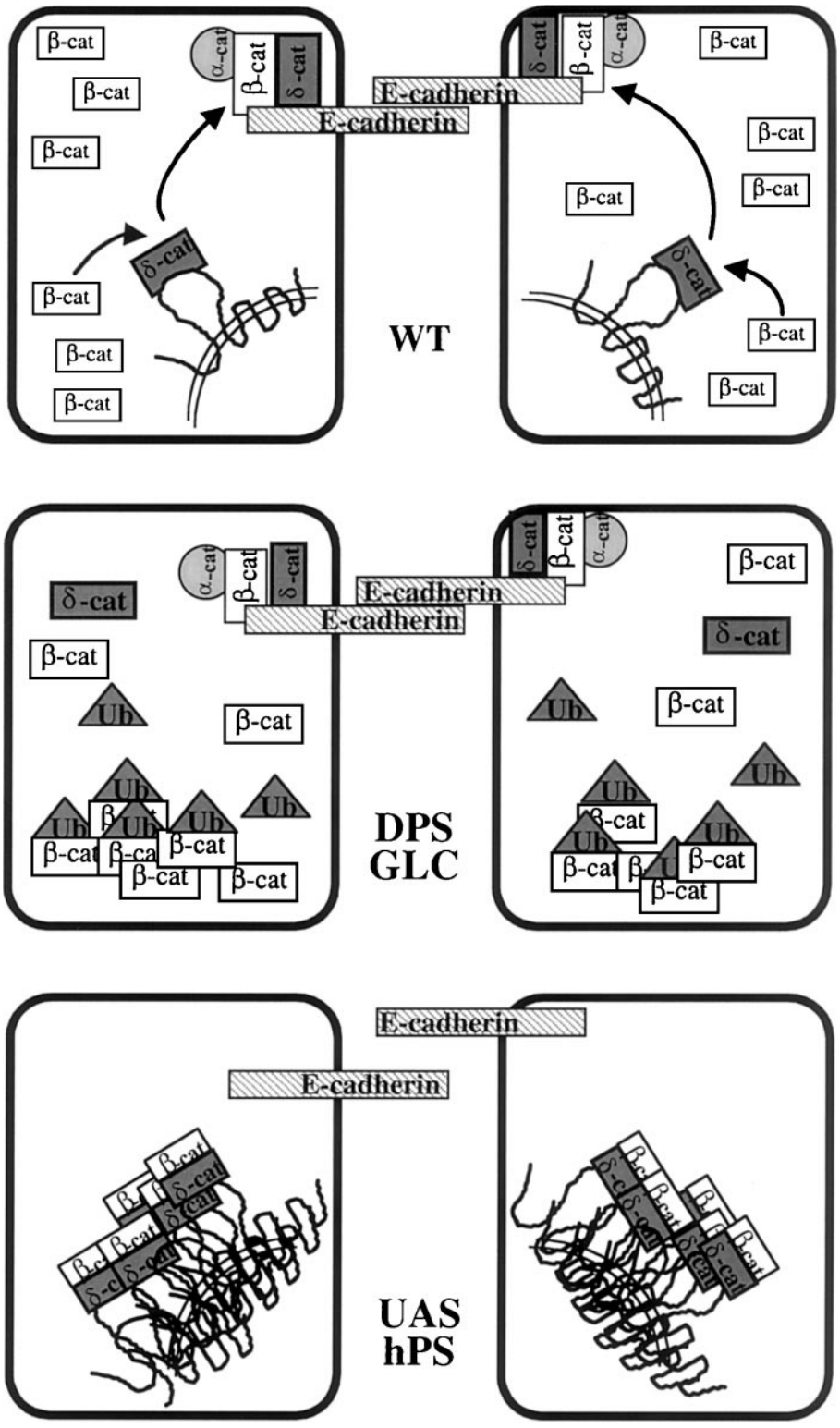


**FIG. 6.** Model of the role of PS1 in Arm/*β*-catenin-mediated cell adhesion. (A) In wild-type, *δ*-catenin binds to the loop region of PS1 and to free *β*-catenin in the cytoplasm. This interaction facilitates efficient translocation of *β*-catenin to the apical adherens junctions, where it then associates with E-cadherin to mediate cell adhesion. (B) In mutants lacking PS1, *β*-catenin is not shuttled efficiently to the apical surface but sufficient Arm is present to reach the apically located adherens junctions and mediate normal cell adhesion via E-cadherin. However, much of the cytoplasmic Arm that would normally be degraded in proteosomes is found in large, Ubiquitin-positive inclusions in the cytoplasm. (C) When PS1 or the PS1 loop is overexpressed at very high levels, it is retained in the ER in association Arm/*β*-catenin and normal trafficking to the adherens junctions fails. This defect renders E-cadherin nonfunctional and disrupts cell adhesion.

tionship between DPS and Arm was also suggested by a genetic modifier screen for mutations which can suppress the *armadillo* mutant phenotype (Cox *et al.,* 2000). Together these observations implicate PS in a complex with β-catenin as a means to target β-catenin and possibly its cargo for degradation or other functions at remote sites in the cell (Fig. 6). Thus the Presenilin/β-catenin complex may serve as an endoplasmic reticular staging platform for complex assembly and targeting to a variety of cellular destinations including the proteosome.

Before β-catenin joins α-catenin and arrives at the plasma membrane, it forms a "preadhesion" complex with Cadherin (Hinck *et al.,* 1994) which is required for ER exit and membrane delivery of the complex (Chen *et al.,* 1999). The delivery of Arm requires that the cell specify a polar trafficking route to the site of the adherens junctions at the apical part of the cell during blastoderm stages. That many of these inclusions were located basally suggests impaired apical trafficking in the absence of PS. Although apparently reduced, sufficient Arm does reach the adherens junctions in these embryos so they do not develop an early adhesion defect phenotype. Instead, the phenotype includes a neurogenic defect thought to be related to the role of PS in cleaving Notch to generate an active product (Struhl and Greenwald, 1999; Ye *et al.,* 1999). The reports of the close resemblance between the Notch and PS phenotypes suggested a highly restricted function for PS: enhancement of Notch function by facilitating Notch cleavage. However, loss of Notch does not produce the Arm inclusions observed with loss of PS (Fig. 1M). This finding suggests a broader function for PS which extends beyond its role in Notch processing.

Expression of hPS1 *in vivo* in the developing *Drosophila* embryo also affected the localization of Armadillo. Using two different GAL4 drivers that are expressed maternally, we observed a *shotgun*-like phenotype in the embryos. Mutations in *shotgun,* which encodes *Drosophila* E-cadherin, have a distinctive loss of head structures and dorsal open cuticle phenotype, due to a loss of cell adhesion caused by nonfunctional adherens junctions. Further, *Armadillo* mutant germ cells have a nearly identical phenotype as *shotgun* mutant germ cells (White *et al.,* 1998). The expression of full-length PS1 or the PS1 loop region alone reduced the amount of Armadillo associated with the cell membrane and increased the cytoplasmic pool of Armadillo. The most likely explanation for why overexpression of hPS1 caused these changes in the intracellular localization of Arm is due to titration of Arm-repeat family members away from the adherens junction or from a complex destined for the adherens junction. In particular, we observed that there was a reduction in the amount of Armadillo associated with the cell membrane. The association of Arm with hPS1 *in vivo* sufficiently reduces Arm in the adherens junction and thereby renders E-cadherin nonfunctional leading to an adhesion defect and lethality in the *Drosophila* embryo.

### *The PS Complex with β-Catenin and p120$^{ctn}$ Subfamily Members*

Both PS1 and β-catenin are found in high-molecular-weight intracellular complexes (Yu *et al.,* 1998); however, they do not directly interact. The identity of at least one other member of the PS/β-catenin complex is δ-catenin. δ-Catenin belongs to an Arm-repeat subfamily structurally defined by a characteristic organization of 10 Arm repeats in the central portion of the protein. Through an interactive domain involving the cytoplasmic loop, PS1 forms a complex with members of the p120$^{ctn}$ subfamily. Among these family members, interactions with PS1 have been demonstrated for δ-catenin (Levesque *et al.,* 1999; Stahl *et al.,* 1999; Tanahashi and Tabira, 1999; Zhou *et al.,* 1997) and p0071 (Stahl *et al.,* 1999). At least two members of the p120$^{ctn}$ subfamily—p120 and δ-catenin—bind to a juxtamembrane site on cadherin which is distinct from the carboxy terminal site where β-catenin binds (Lu *et al.,* 1999; Ozawa and Kemler, 1998; Yap *et al.,* 1998). Without either of these binding domains the extracellular region of E-cadherin can confer some adhesive properties to cells; however, these cells do not undergo compaction, e.g., change in cell shape to maximize contact area (Ozawa and Kemler, 1998). Deletion of the β-catenin binding domain of E-cadherin while retaining the juxtamembrane domain causes loss of adhesion. Constructs with a retained juxtamembrane domain are also incapable of forming E-cadherin dimers, a key step by which cells acquire adhesive properties. These data have led some to conclude that binding to the juxtamembrane region of classical cadherins is inhibitory to cell adhesion (Ozawa and Kemler, 1998).

The binding studies here suggest that the association of PS and β-catenin occurs through δ-catenin. Among the p120$^{ctn}$ subfamily proteins, the expression of δ-catenin is highly restricted to neurons (Ho *et al.,* 2000). Other family members, such as p0071 and p120$^{ctn}$, are more broadly expressed, and they may attract β-catenin to PS in nonneuronal cells. Because excess PS can interfere with the delivery of β-catenin to the adherens junction, one possible function of the PS/β-catenin complex is to coordinate the delivery of β-catenin with a 10-repeat Arm protein to the adherens junctions.

### *The PS/β-Catenin Complex and Notch*

One site of residence for β-catenin is in a complex with Axin, APC, and GSK3β where it mediates regulation of Wnt signaling. Although quantitatively less frequent than the *shotgun* phenotype among embryos expressing hPS1, phenotypes that resemble the loss *wingless* activity were occasionally observed suggesting that binding to hPS1 also successfully competed β-catenin away from its signaling pool. In conjunction with the evidence that PS is involved in Notch activation by releasing its cytoplasmic domain (De Strooper *et al.,* 1999; Struhl and Greenwald, 1999; Ye *et al.,* 1999) these findings suggest another link between the Wingless and Notch pathways. Previous studies reported

genetic interactions between *wg* and *N* (Couso and Martinez Arias, 1994; Hing *et al.*, 1994) and direct interactions between these pathways via Dishevelled (Axelrod *et al.*, 1996), as well as isolation of *wg* mutations in screens for genetic modifiers of Notch (Hing *et al.*, 1994) and vice versa (Couso and Martinez Arias, 1994).

PS is primarily localized in the ER, but cleavage of Notch occurs either at or close to the cell surface. Transit of PS to the region of the adherens junction, as recently observed (Georgakopoulos *et al.*, 1999), could resolve the contradiction between previous views regarding the location of PS in the endoplasmic reticulum and the cleavage of Notch either in or near the plasma membrane. PS is associated with two proteins—β-catenin and δ-catenin—whose destination is the adherens junction. Both Notch and Wingless have also been reported in the region of the adherens junction (Woods and Bryant, 1993). A large regulatory complex associated with PS may cleave Notch leaving the released cytoplasmic fragment to translocate to the nucleus and activate transcription or prevent transcription by binding through its carboxy terminus to Dsh. Dishevelled may independently localize to intracellular junctions through its disks large homology (DHR) region or utilize the PS complex to direct the Notch cytoplasmic fragment toward a degradative pathway. Alternatively, members of the PS complex such as β- or δ-catenin may regulate the inhibitory interaction between Notch and Dishevelled. Because both of the putative substrates for PS—Notch and the amyloid precursor protein—transit through the ER, PS-associated proteins may serve in the ER to prevent premature cleavage. If, as suggested (De Strooper *et al.*, 1999; Struhl and Greenwald, 1999; Ye *et al.*, 1999), PS cleaves Notch, it is curious that the expression of hPS1 did not induce a Notch activation phenotype. Neither was a Notch activation phenotype reported when the endogenous DPS was overexpressed (Guo *et al.*, 1999; Ye *et al.*, 1999). Significant inhibitory controls must be present that either prevent Notch cleavage or prevent the activity of the Notch active fragment.

The interactions of β-catenin with other proteins are complex and numerous. PS1 may delimit the components of the β-catenin complex at specific cellular locales and allow it to discriminate among potential binding partners. In the case of the β-catenin/APC complex, GSK3β can phosphorylate both proteins (Rubinfeld *et al.*, 1996). Although a direct association of GSK3β with β-catenin could not be demonstrated *in vitro*, it has been observed that Axin simultaneously and directly binds to APC, β-catenin, and GSK3β (Hart *et al.*, 1998; Ikeda *et al.*, 1998). The binding of all three proteins to axin may coordinate β-catenin downregulation by bringing these proteins into proximity. GSK3β binds PS1 between residues 259 and 298 of the fragment which is N-terminal after endoproteolytic cleavage (Takashima *et al.*, 1998). This site differs from the δ-catenin binding site on hPS1 which spans residues 319 to 371 (M. Medina and K. S. Kosik, unpublished data). Thus PS may coordinate the entry of both β-catenin and GSK3β into the complex with APC and Axin. Alternatively, PS may coordinate the trafficking routes of β-catenin as it assembles and shifts large multicomponent protein complexes to diverse destinations in the cell.

## MATERIALS AND METHODS

### *Drosophila Expression Constructs, GAL-4 Lines, and Germline Clones*

cDNAs encoding human wild-type *PS1* and the *PS1* loop region (amino acids 263–407) were ligated into pUAST (Brand and Perrimon, 1993). Transgenic lines were generated by injection of CsCl banded DNA at a concentration of 600 μg/ml into *yw; △2.3/TM6* embryos, which contain the P-element transposase. Multiple insertions of each construct were isolated and two insertions of each construct were analyzed. The insertion *yw;P[w$^+$,UAS-PS1$^{wtD2}$]* on chromosome II is homozygous viable, the insertion *yw;P[w$^+$,UAS-PS1$^{wtD4}$]/Ly* on chromosome III is semiviable, and two insertions of the loop region (*yw; P[w$^+$, UAS-PS1-loopA]* and *yw; P[w$^+$, UAS-PS1-loopA8]*) are both homozygous viable insertions on chromosome II.

Males homozygous for the UAS constructs were crossed with flies carrying GAL4 insertions under the control of one of two maternal promoters. *yw;P[w$^+$, Gal4$^{U32a}$]* is a fusion of the α4-tubulin promoter and the VP16 activation domain driving GAL-4. This construct is inserted on chromosome II and is homozygous viable (generously provided by D. St. Johnston). The *nanos-GAL4* line, a viable insertion on chromosome III, drives expression of GAL-4 under the control of the *nanos* promoter (generously provided by P. Rorth).

Two alleles of PS1 were examined in germline clones (*PS$^{C1}$-FRT* and *PS$^{C2}$-FRT;* Struhl and Greenwald, 1999) generated using the FLP-FRT method (Perrimon, 1996).

Collections of *Drosophila* embryos were prepared according to standard methods. Briefly, embryos were dechorionated (50% bleach) and rinsed in 0.1% Triton. Embryos were fixed for 10–15 min in a 1:1 solution of 4% methanol-free formaldehyde (Polysciences) in phosphate-buffered saline (PBS) and heptane (Sigma) on a rotator. Formaldehyde phase was then removed and replaced by methanol and then shaken to remove vitelline membranes. All liquid was removed (along with floating embryos) and embryos were briefly rinsed in methanol. They were then rehydrated in PT (0.1% Tween 20 in 1× PBS) three times. PT was aspirated and a solution 1:1 of PT, normal horse serum, and 0.1% NP-40 (Roche–Boehringer Mannheim) was added. Embryos were incubated in this solution on a rotator for at least 30 min at room temperature (RT). Anti-Armadillo antibody (Developmental Hybridoma Bank, Iowa City, IA) or anti-E-cadherin antibody (generously provided by generously provided by T. Uemura) was diluted 1:10 to 1:20 in 0.1% NP-40/PBS and incubated at RT for 2 h, rinsed four times in PT, incubated in horse anti-mouse FITC (1:500) in PT for 2 h at RT, and rinsed again four times in PT. Embryos were then suspended in SlowFade (Molecular Probes) suspended in glycerol. Staining was visualized on a Leica confocal microscope.

For visualization of F-actin, embryos were fixed as described above and devitellinized using 95% ethanol instead of methanol. Embryos were then rinsed in 95% ethanol and rehydrated in PT. Texas red-conjugated phalloidin was suspended in PT and embryos were mounted in SlowFade suspended in glycerol. Cuticle preparations were performed as described previously (Struhl, 1985).

### *Yeast Two-Hybrid System*

The indicated portions of human PS1 were PCR amplified and cloned into either pAS2-1 (to produce a GAL4 binding domain fusion protein) or pACT2 (to produce a GAL4 transactivation domain fusion protein). Mouse β-catenin deletion constructs were prepared by standard cloning techniques. All constructs were confirmed by sequencing. The Matchmaker GAL4 two-hybrid system (Clontech Laboratories) was used according to the manufacturer's instructions. The yeast strain Y187 was transformed using the LiAc transformation procedure and cotransformants were selected on -Leu;-Trp medium. To look for specific interactions, 8–10 double transformants colonies were assayed on filters for β-galactosidase activity and also grown in -Leu;-Trp;-His medium. For quantification, liquid β-galactosidase assays were carried out on four to six independent colonies in triplicate for each plasmid combination tested. β-Galactosidase activity was calculated in Miller units (Miller, 1972).

### *Transfections*

CHO–PS70 cells (Xia *et al.,* 1997) were grown in 10-cm dishes and transfected with 10 μg of plasmid DNA using Superfect (Qiagen). Cells were collected 72 h after transfection, washed three times in cold PBS, and resuspended in lysis buffer (1% NP-40; 12.5 mM Chaps; 150 mM NaCl; 50 mM Tris, pH 7.6; 2 mM EDTA; 1 mM PMSF; Complete protease inhibitors). After incubation at 4°C for 30 min, lysates were spun for 15 min in the microfuge and the supernatants were stored at −80°C until use. Protein concentration was determined by the BCA assay (Pierce).

### *Immunoprecipitation and Immunoblot*

The lysates (1 mg of total protein) were precleared by incubating with protein G–Sepharose beads (Pharmacia) for 1 h at 4°C followed by centrifugation to remove the beads. The supernatants were immunoprecipitated with specific antibodies overnight at 4°C. Polyclonal antibodies against β-catenin (Transduction Laboratories), PS1 (X81 (Xia *et al.,* 1997), R-4873 (Zhou *et al.,* 1997), and δ-catenin (rAb62 (Lu *et al.,* 1999)) were used. The immunoprecipitates were then captured by protein G–Sepharose for an additional 1 h at 4°C. Samples were washed four times with IP buffer (15 mM Tris, pH 7.5; 5 mM EDTA; 2.5 mM EGTA; 1% Triton X-100; 0.1% SDS; 120 mM NaCl; and 25 mM KCl). Immunoprecipitates were resuspended in 2× Laemmli buffer, boiled for 5 min, separated by SDS–PAGE, and transferred to nitrocellulose membranes (PGC Scientifics) for immunoblotting. Protein was detected by enhanced chemiluminescence (ECL) (Amersham).

An equal number of wild-type and germline clones (24 embryos of clone C1 and 100 embryos of clone C2) were collected and dechorionated using 50% bleach solution. Embryos were washed in $dH_2O$, transferred to an Eppendorf tube, and dounced in 500 μl of RIPA buffer (150 mM NaCl; 50 mM Tris, pH 8.0; 1% NP-40; 0.5% deoxycholic acid; 0.1% SDS) supplemented with a protease inhibitor cocktail (Boehringer Mannheim). Lysates were spun at 14,000 rpm for 20 min and the supernatant was stored at −20°C until use. Samples were immunoblotted as above using the anti-Armadillo antibody 7A1 (Developmental Hybridoma Bank,).

### *In Vitro Binding Assays*

Generation of recombinant GST fusion proteins containing the N-terminus (corresponding to amino acids 1–81) (GST-N) and the hydrophilic loop region (amino acids 267–407) (GST-L) has been described previously (Zhou *et al.,* 1997). Wild-type *Drosophila* embryos (0–3 h) were homogenized in lysis buffer (1% NP-40; 50 mM Tris, pH 7.6; 150 mM NaCl; 2 mM EDTA; 1 mM PMSF; Complete protease inhibitors) and the lysate was stored at −80°C until use. Pulldown assays were carried out as described previously (Zhou *et al.,* 1997).

The TnT-coupled transcription/translation reticulocyte lysate system (Promega) was used to translate [$^{35}$S]methionine-labeled proteins *in vitro*. Translated products were mixed together in *in vitro* binding (IVB) buffer (10 mM Tris–HCl pH 8.0; 150 mM NaCl; 5 mM EDTA; 0.2% NP-40; 1 mM DTT; 1 mM PMSF; Complete protease inhibitors) and precleared with protein G–Sepharose beads for 1 h at 4°C. After centrifugation, the corresponding antibody was added to the supernatants and incubated at 4°C for an additional 1 h. Immunoprecipitates were washed four times with IVB buffer, resuspended in Laemmli buffer, and separated by SDS–PAGE. Gels were treated with Amplify (Amersham), dried, and exposed.

## REFERENCES


Aberle, H., Butz, S., Stappert, J., Weissig, H., Kemler, R., and Hoschuetzky, H. (1994). Assembly of the cadherin–catenin complex in vitro with recombinant proteins. *J. Cell Sci.* **107,** 3655–3663.

Axelrod, J. D., Matsuno, K., Artavanis-Tsakonas, S., and Perrimon, N. (1996). Interaction between Wingless and Notch signaling pathways mediated by dishevelled. *Science* **271,** 1826–1832.

Borchelt, D. R., Thinakaran, G., Eckman, C. B., Lee, M. K., Davenport, F., Ratovitsky, T., Prada, C.-M., Kim, G., Seekins, S., Yager, D., Slunt, H. H., Wang, R., Seeger, M., Levey, A. I., Gandy, S. E., Copeland, N. G., Jenkins, N. A., Price, D. L., Younkin, S. G., and Sisodia, S. S. (1996). Familial Alzheimer's disease-linked presenilin 1 variants elevate Aβ1–42/1–40 ratio in vitro and in vivo. *Neuron* **17,** 1005–1013.

Brand, A. H., and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118,** 401–415.

Brunner, E., Peter, O., Schweizer, L., and Basler, K. (1997). Pangolin encodes a Lef-1 homologue that acts downstream of Armadillo to transduce the Wingless signal in *Drosophila*. *Nature* **385,** 829–833.

Chen, Y.-T., Stewart, D. B., and James Nelson, W. J. (1999). Coupling assembly of the E-cadherin/β-catenin complex to efficient endoplasmic reticulum exit and basal-lateral membrane targeting of E-cadherin in polarized MDCK cells. *J. Cell Biol.* **144,** 687–699.

Citron, M., Westaway, D., Xia, W., Carlson, G., Diehl, T., Levesque, G., Johnson-Wood, K., Lee, M., Seubert, P., Davis, A., Kholodenko, D., Motter, R., Sherrington, R., Perry, B., Yao, H., Strome, R., Lieberburg, I., Rommens, J., Kim, S., Schenk, D., Fraser, P., St. George-Hyslop, P., and Selkoe, D. (1997). Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid β-protein in both transfected cells and transgenic mice. *Nature Med.* **3,** 67–72.

Conlon, R., Reaume, A., and Rossant, J. (1995). Notch 1 is required for the coordinate segmentation of somites. *Development* **121,** 1533–1545.

Couso, J. P., and Martinez Arias, A. (1994). Notch is required for wingless signaling in the epidermis of Drosophila. *Cell* **79,** 259–272.

Cox, R., McEwen, D., Myster, D., Duronio, R., Loureiro, J., and Peifer, M. (2000). A screen for mutations that supress the phenotype of *Drosophila armadillo*, the β-catenin homolog. *Genetics*, in press.

De Strooper, B., Annaert, W., Cupers, P., Saftig, P., Craessaerts, K., Mumm, J. S., Schroeter, E. H., Schrijvers, V., Wolfe, M. S., Ray, W. J., Goate, A., and Kopan, R. (1999). A presenilin-1-dependent gamma-secretase-like protease mediates release of Notch intracellular domain. *Nature* **398,** 518–22.

De Strooper, B., Saftig, P., Craessaerts, K., Vanderstichele, H., Guhde, G., Annaert, W., Von Figura, K., and Van Leuven, F. (1998). Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. *Nature* **391,** 387–390.

Donoviel, D. B., Hadjantonakis, A.-K., Ikeda, M., Zheng, H., St. George-Hyslop, P., and Bernstein, A. (1999). Mice lacking both presenilin genes exhibit early embryonic patterning defects. *Genes Dev.* **13,** 2801–2810.

Duff, K., Eckman, C., Zehr, C., Yu, X., Prada, C.-M., Perez-tur, M. J., Hutton, M., Buee, L., Harigaya, Y., Morgan, D., Gordon, M. N., Holcomb, L., Refoloe, L., Zenk, B., Hardy, J., and Younkin, S. (1996). Increased amyloid Aβ42(43) in brains of mice expressing mutant presenilin 1. *Nature* **383,** 710–713.

Esler, W., Kimberly, W., Ostaszewski, B., Diehl, T., Moore, C., Tsai, J., Rahmati, T., Xia, W., Selkoe, D., and Wolfe, M. (2000). Transition-state analogue inhibitors of γ-secretase bind directly to presenilin-1. *Nature Cell Biol.* **2,** 428–434.

Georgakopoulos, A., Marambaud, P., Efthimiopoulos, S., Shioi, J., Cui, W., Li, H.-C., Schütte, M., Gordon, R., Holstein, G. R., Martinelli, G., Mehta, P., Friedrich, V. L., Jr., and Robakis, N. K. (1999). Presenilin-1 forms complexes with the cadherin/catenin cell–cell adhesion system and is recruited to intercellular and synaptic contacts. *Mol. Cell* **4,** 893–902.

Guo, Y., Livne-Bar, I., Zhou, L., and Boulianne, G. L. (1999). *Drosophila* presenilin is required for neuronal differentiation and affects notch subcellular localization and signaling. *J. Neurosci.* **19,** 8435–8442.

Hart, M. J., de los Santos, R., Albert, I. N., Rubinfeld, B., and Polakis, P. (1998). Downregulation of beta-catenin by human Axin and its association with the APC tumor suppressor, beta-catenin and GSK3 beta. *Curr. Biol.* **8,** 573–581.

Hatzfeld, M., and Nachtsheim, C. (1996). Cloning and characterization of a new armadillo family member, P0071, associated with the junctional plaque-evidence for a subfamily of closely related proteins. *J. Cell Sci.* **109,** 2767–2778.

Herreman, A., Serneels, L., Annaer, t. W., Collen, D., Schoonjans, L., and De Strooper, B. (2000). Total inactivation of gamma-secretase activity in presenilin-deficient embryonic stem cells. *Nature Cell Biol.* **2,** 461–462.

Hinck, L., Nathke, I. S., Papkoff, J., and Nelson, W. J. (1994). Dynamics of cadherin/catenin complex formation: Novel protein interactions and pathways of complex assembly. *J. Cell Biol.* **125,** 1327–1340.

Hing, H. K., Sun, X., and Artavanis-Tsakonas, S. (1994). Modulation of wingless signaling by Notch in *Drosophila*. *Mech. Dev.* **47,** 261–268.

Ho, C., Zhou, J., Medina, M., Goto, T., Jacobson, M., Bhide, P., and Kosik, K. S. (2000). Expression of the neuronal specific adherens junction protein δ-catenin. *J. Comp. Neurol.* **420,** 261–276.

Ikeda, S., Kishida, S., Yamamoto, H., Murai, H., Koyama, S., and Kikuchi, A. (1998). Axin, a negative regulator of the Wnt signaling pathway, forms a complex with GSK-3beta and beta-catenin and promotes GSK-3beta-dependent phosphorylation of beta-catenin. *EMBO J.* **17,** 1371–1384.

Jiang, J., and Struhl, G. (1998). Regulation of the Hedgehog and Wingless signalling pathways by the F-box/WD40-repeat protein Slimb. *Nature* **391,** 493–496.

Kang, D. E., Soriano, S., Frosch, M. P., Collins, T., Naruse, S., Sisodia, S. S., Leibowitz, G., Levine, F., and Koo, E. H. (1999). Presenilin 1 facilitates the constitutive turnover of beta-catenin: Differential activity of Alzheimer's disease-linked PS1 mutants in the beta-catenin-signaling pathway. *J. Neurosci.* **19,** 4229–1437.

Knust, E. (1996). *Drosophila* morphogenesis: Follow-my-leader in epithelia. *Curr. Biol.* **6,** 379–381.

Lemere, C. A., Lopera, F., Kosik, K. S., Lendon, C. L., Ossa, J., Saido, T. C., Yamaguchi, H., Ruiz, A., Martinez, A., Madrigal, L., Hincapie, L., Arango L, J. C., Anthony, D. C., Koo, E. H., Goate, A. M., Selkoe, D. J., and Arango V, J. C. (1996). The E280A presenilin 1 Alzheimer mutation produces increased Aβ42 deposition and severe cerebellar pathology. *Nature Med.* **2,** 1146–1148.

Levesque, G., Yu, G., Nishimura, M., Zhang, D. M., Levesque, L., Yu, H., Xu, D., Liang, Y., Rogaeva, E., Ikeda, M., Duthie, M., Murgolo, N., Wang, L., VanderVere, P., Bayne, M. L., Strader, C. D., Rommens, J. M., Fraser, P. E., and St. George-Hyslop, P. (1999). Presenilins interact with armadillo proteins including neural-specific plakophilin-related protein and beta-catenin. *J. Neurochem.* **72,** 999–1008.

Levitan, D., and Greenwald, I. (1995). Facilitation of lin 12-mediated signalling by sel-12, a *Caernorhabiditis elegans* S182 Alzheimer's disease gene. *Nature* **377,** 351–354.

Li, Y., Xu, M., Lai, M., Huang, Q., Castro, J., DiMuzio-Mower, J., Harrison, T., Lellis, C., Nadin, A., Neduvelil, J., Registe, R. R., Sardana, M., Shearman, M., Smith, A., Shi, X., Yin, K., Shafer, J., and Gardell, S. (2000). Photoactivated gamma-secretase inhibitors directed to the active site covalently label presenilin 1. *Nature* **405,** 689–694.

Lu, Q., Paredes, M., Medina, M., Zhou, J., Cavallo, R., Peifer, M., Orecchio, L., and Kosik, K. (1999). δ-Catenin, an adhesive junction associated protein which promotes cell scattering. *J. Cell Biol.* **144,** 519–532.

Mann, D. M., Iwatsubo, T., Cairns, N. J., Lantos, P. L., Nochlin, D., Sumi, S. M., Bird, T. D., Poorkaj, P., Hardy, J., Hutton, M., Prihar, G., Crook, R., Rossor, M. N., and Haltia, M. (1996). Amyloid beta protein (Aβ) deposition in chromosome 14-linked Alzheimer's disease: Predominance of Aβ42 (43). *Ann. Neurol.* **40,** 149–156.

Medina, M., Marinescu, R. C., Overhauser, J., and Kosik, K. S. (2000). Hemizygosity of δ-catenin (CTNND2) is associated with severe mental retardation in Cri-du-Chat syndrome. *Genomics* **63,** 157–164.

Miller, J. H. (1972). "Experiments in Molecular Genetics." Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Murayama, M., Tanaka, S., Palacino, J., Murayama, O., Honda, T., Sun, X., Yasutake, K., Nihonmatsu, N., Wolozin, B., and Takashima, A. (1998). Direct association of presenilin-1 with beta-catenin. *FEBS Lett.* **433,** 73–77.

Nishimura, N., Yu, G., Levesque, G., Zhang, D. M., Ruel, L., Chen, F., Milman, P., Holmes, E., Liang, Y., Kawarai, T., Jo, E., Supala, A., Rogaeva, E., Xu, D.-M., Janus, C., Levesque, L., Bi, Q., Duthie,

M., Rozmahek, R., Mattila, K., Lannfelt, L., Westaway, D., Mount, H. T. J., Woodgetti, J., Fraser, P., and St. George-Hyslop, P. (1999). Presenilin mutations associated with Alzheimer's disease cause defective intracellular trafficking of β-catenin, a component of the presenilin protein complex. *Nature Med.* **5,** 164–169.

Oyama, T., Kanai, Y., Ochiai, A., Akimoto, S., Oda, T., Yanagihara, K., Nagafuchi, A., Tsukita, S., Shibamoto, S., Ito, F., *et al.* (1994). A truncated beta-catenin disrupts the interaction between E-cadherin and alpha-catenin: A cause of loss of intercellular adhesiveness in human cancer cell lines. *Cancer Res.* **54,** 6282–6287.

Ozawa, M., and Kemler, R. (1998). The membrane-proximal region of the E-cadherin cytoplasmic domain prevents dimerization and negatively regulates adhesion activity. *J. Cell Biol.* **142,** 1605–1613.

Peifer, M., Berg, S., and Reynolds, A. B. (1994). A repeating amino acid motif shared by proteins with diverse cellular roles. *Cell* **76,** 789–791.

Peifer, M., Rauskolb, C., Williams, M., Riggleman, B., and Wieschaus, E. (1991). The segment polarity gene armadillo interacts with the wingless signaling pathway in both embryonic and adult pattern formation. *Development* **111,** 1029–1043.

Perrimon, N. (1996). Serpentine proteins slither into the Wingless and Hedgehog fields. *Cell* **86,** 513–516.

Ray, W. J., Ashall, F., and Goate, A. M. (1998). Molecular pathogenesis of sporadic and familial forms of Alzheimer's disease. *Mol. Med. Today* **4,** 151–157.

Ray, W. J., Yao, M., Nowotny, P., Mumm, J., Zhang, W., Wu, J. Y., Kopan, R., and Goate, A. M. (1999). Evidence for a physical interaction between presenilin and Notch. *Proc. Natl. Acad. Sci. USA* **96,** 3263–3268.

Riese, J., Yu, X., Munnerlyn, A., Eresh, S., Hsu, S. C., Grosschedl, R., and Bienz, M. (1997). LEF-1, a nuclear factor coordinating signaling inputs from wingless and decapentaplegic. *Cell* **88,** 777–787.

Rubinfeld, B., Albert, I., Porfiri, E., Fiol, C., Munemitsu, S., and Polakis, P. (1996). Binding of GSK3 beta to the APC–beta-catenin compolex and regulation of comples assembly. *Science* **272,** 1023–1026.

Scheuner, D., Eckman, C., Jensen, M., Song, X., Citron, M., Suzuki, N., Bird, T. D., Hardy, J., Hutton, M., Kukull, W., Larson, E., Levy-Lahad, E., Viitanen, M., Pesking, E., Poorkaj, P., Schellenberg, G., Tanzi, R., Wasco, W., Lannfelt, L., Selkoe, D., and Younkin, S. (1996). Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nature Med.* **2,** 848–870.

Shen, J., Bronson, T., Chjen, D. F., Xia, W., Selkoe, D. J., and Tonegawa, S. (1997). Skeletal and CNS defects in *presenilin-1*-deficient mice. *Cell* **89,** 629–639.

Stahl, B., Diehlmann, A., and Sudhof, T. C. (1999). Direct interaction of Alzheimer's disease-related presenilin 1 with armadillo protein p0071. *J. Biol. Chem.* **274,** 9141–9148.

Struhl, G. (1985). Near-reciprocal phenotypes caused by inactivation or indiscriminate expression of the *Drosophila* segmentation gene ftz. *Nature* **318,** 677–680.

Struhl, G., and Greenwald, I. (1999). Presenilin is required for activity and nuclear access of Notch in *Drosophila. Nature* **398,** 522–525.

Swiatek, P., Lindsell, C., del Amo, F., Weinmaster, G., and Grindley, T. (1994). Notch 1 is essential for postimplantation development in mice. *Genes Dev.* **8,** 707–719.

Takashima, A., Murayama, M., Murayama, O., Kohno, T., Honda, T., Yasutake, K., Nihonmatsu, N., Mercken, M., Yamaguchi, H., Sugihara, S., and Wolozin, B. (1998). Presenilin 1 associates with glycogen synthase kinase-3beta and its substrate tau. *Proc. Natl. Acad. Sci. USA* **95,** 9637–9641.

Tanahashi, H., and Tabira, T. (1999). Isolation of human δ-catenin and its binding specificity with presenilin 1. *NeuroReport* **10,** 563–568.

Tesco, G., Kim, T. W., Diehlmann, A., Beyreuther, K., and Tanzi, R. E. (1998). Abrogation of the presenilin 1/beta-catenin interaction and preservation of the heterodimeric presenilin 1 complex following caspase activation. *J. Biol. Chem.* **273,** 33909–33914.

Thinakaran, G., Borchelt, D. R., Lee, M. K., Slunt, H. H., Spitzer, L., Kim, G., Ratovitsky, T., Davenport, F., Nordstedt, C., Seeger, M., Hardy, J., Levey, A. I., Gandy, S. E., Jenkins, N. A., Copeland, N. G., Price, D. L., and Sisodia, S. S. (1996). Endoproteolysis of presenilin 1 and accumulation of processed derivatives in vivo. *Neuron* **17,** 181–190.

Tomita, T., Maruyama, K., Saido, T. C., Kume, H., Shinozaki, M. K., Tokuhiro, S., Capell, A., Walter, J., Gruenberg, H., Haass, C., Iwatsubo, T., and Obata, K. (1997). The presenilin 2 mutations (N141I) linked to familial Alzheimer's disease (volga German families) increases the secretion of amyloid β-protein ending at the 42nd (or 43rd) residue. *Proc. Natl. Acad. Sci. USA* **94,** 2025–2030.

van de Wetering, M., Cavallo, R., Dooijes, D., van Beest, M., van Es, J., Loureiro, J., Ypma, A., Hursh, D., Jones, T., Bejsovec, A., Peifer, M., Mortin, M., and Clevers, H. (1997). Armadillo coactivates transcription driven by the product of the *Drosophila* segment polarity gene dTCF. *Cell* **88,** 789–99.

White, P., Aberle, H., and Vincent, J. P. (1998). Signaling and adhesion activities of mammalian beta-catenin and plakoglobin in *Drosophila. J. Cell Biol.* **140,** 183–195.

Wolfe, M. S., Moore, C. L., Xia, W., Leatherwood, D. D., Donkor, I. O., and Selkoe, D. J. (1998). Peptidomimetic probes for Alzheimer's g-secretases. *Soc. Neurosci. Abstr.* **24**(Pt. 1), 1005 (399.5).

Wong, P., Zheng, H., Chen, H., Becher, M., Sirinathsinghji, D., Trumbauer, M., Chen, H., Price, D., Van fer Ploeg, L., and Sisodia, S. (1997). Presenilin 1 is required for Notch 1 and Dll 1 expression in the paraxial mesoderm. *Nature* **387,** 288–292.

Woods, D. F., and Bryant, P. J. (1993). Apical junctions and cell signalling in epithelia. *J. Cell Sci. Suppl.* **17,** 171–183.

Wu, G., Hubbard, E. J. A., Kitajewski, J. K., and Greenwald, I. (1998). Evidence for functional and physical association between *Caenorhabditis elegans* SEL-10, a Cdc4p-related protein, and SEL-12 presenilin. *Proc. Natl. Acad. Sci. USA* **95,** 15787–15791.

Xia, W., Zhang, J., Kholodenko, D., Citron, M, Podlisny, M. B., Teplow, D. B., Haass, C., Seubert, P., Koo, E. H., and Selkoe, D. J. (1997). Enhanced production and oligomerization of the 42-residue amyloid β-protein by Chinese hamster ovary cells stably expressing mutant presenilins. *J. Biol. Chem.* **272,** 7977–7982.

Yap, A. S., Niessen, C. M., and Gumbiner, B. M. (1998). The juxtamembrane region of the cadherin cytoplasmic tail supports

lateral clustering, adhesive strengthening, and interaction with p120. *J. Cell Biol.* **141,** 779–789.

Ye, Y., Lukinova, N., and Fortini, M. E. (1999). Neurogenic phenotypes and altered Notch processing in *Drosophila* Presenilin mutants. *Nature* **398,** 525–529.

Young, P. E., Pesacreta, T. C., and Kiehart, D. P. (1991). Dynamic changes in the distribution of cytoplasmic myosin during *Drosophila* embryogenesis. *Development* **111,** 1–14.

Young, P. E., Richman, A. M., Ketchum, A. S., and Kiehart, D. P. (1993). Morphogenesis in *Drosophila* requires nonmuscle myosin heavy chain function. *Genes Dev.* **7,** 29–41.

Yu, G., Chen, F., Levesque, G., Nishimura, M., Zhang, D. M., Levesque, L., Rogaeva, E., Xu, D., Liang, Y., Duthie, M., St. George-Hyslop, P. H., and Fraser, P. E. (1998). The presenilin 1 protein is a component of a high molecular weight intracellular complex that contains beta-catenin. *J. Biol. Chem.* **273,** 16470–16475.

Zhang, Z., Hartmann, H., Do, V. M., Abramowski, D., Sturchler-Pierrat, C., Staufenbiel, M., Sommer, B., va de Wetering, M., Clevers, H., Saftig, P., De Strooper, B., He, X., and Yankner, B. (1998). Destabilization of β-catenin by mutations in presenilin-1 potentiates neuronal apoptosis. *Nature* **395,** 698–702.

Zhang, Z., Nadeau, P., Song, W., Donoviel, D., Yuan, M., Bernstein, A., and Yankner, B. (2000). Presenilins are required for gamma-secretase cleavage of beta-APP and transmembrane cleavage of Notch-1. *Nature Cell Biol.* **2,** 463–465.

Zhou, J., Liyanage, U., Medina, M., Ho, C., Simmons, A. D., Lovett, M., and Kosik, K. S. (1997). Presenilin 1 interacts in brain with a novel member of the armadillo family. *NeuroReport* **8,** 2085–2090.

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